Iranian Journal of Veterinary Science and Technology
Vol. 7, No. 2, 2015, 75-83

IJVST

Morphologic and morphometric variations of the adult and the eggs of frequent *Fasciola* species from domestic ruminants of North West of Iran

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Received:April 20,2014 Accepted: August 13, 2016

Abstract

Worldwide including Iran, *Fasciola* species are the causes of human and animal fasciolosis which have comparatively identical morphologies. The present study is aimed to identify different *Fasciola* forms by using morphologic and morphometric variations from domestic ruminants of northwestern Iran. A total of 130, 67 and 140 livers of cattle, water buffaloes and sheep respectively were collected from Urmia slaughterhouse. The adult helminths were removed and stained using Acetocarmine staining. The *Fasciola* eggs were directly extracted from the uterus of adult helminths. The overall frequency of infection was 28.19%. The highest infection rate was found in water buffalos (34%). The highest number of helminth per each animal was recorded for cattle (9.23%). The predominant infecting fasciolid in the examined ruminants was *F. gigantica* (51.89%) from water buffaloes origin. There was a significant difference among the width (W), the length (L), and the distance between ventral sucker to the posterior end of the body (DBVE) of all *Fasciola* forms from the examined animals. The L of different *Fasciola* forms had a significant difference for *F. gigantica* and intermediate form of *Fasciola* from cattle and water buffaloes origins. The W and the proportion of the body length to the width (SI) of eggs from all *Fasciola* forms had no significant difference. The Ls of eggs of *F. hepatica* and *F. gigantica* were significantly different. The results of this study elucidated three forms of *Fasciola* co-existing in the ruminants of the region. Additionally, the morphology and morphometry of adult and eggs of *Fasciola* species within a range of hosts may be taxonomically informative and one of the character sets for discrimination of fasciolid forms.

Key words: *Fasciola*; Egg; Ruminant, Iran

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Introduction

Fasciolosis is a helminth born parasite disease worldwide which is remarkable in the region (Imani-Baran *et al.*, 2012). The disease is an old parasitic infection (Kendall and McCullough, 1951). In Europe, it was believed that the presence of infection dates back to the Mesolithic and Neolithic periods, namely 5000-5100 years ago (Mas-Coma, 2003). Classically, the prevalence of fasciolosis has a "Spotty distribution" style. However, in spite of the old age of the disease and considering the economic damages which it imposes on animal husbandry industry directly or indirectly, around 2.4 million people in the world are affected by this parasite and about 80 million people are at risk of infection (Massoud, 1974). In terms of Veterinary, many domesticated herbivorous animals become affected by metabolic and liver disorders due to being infected with the parasite, and they will not have any economic efficiency (Ghobadi and Yakhchali, 2003, Yakhchali and Ghobadi, 2005, Eslami, 2008).

Fasciola is a prevalent trematode of bile ducts which is reported from humans and a range of animals, i.e. cattle, buffalo, sheep, goat, donkey, horse, rabbit, pig (Rim *et al.*, 1994, Salahi-Moghaddam, 2004). The presence of the intermediate form of *Fasciola* in east Mediterranean countries was an issue (Farag *et al.*, 1979). In Asia, especially in Japan, Taiwan, Philippines and South Korea, morphologically, *Fasciola* species showed remarkable variations (Watanabe, 1962, Oshima *et al.*, 1968, Akahane *et al.*, 1970, Kimura *et al.*, 1984, Srimuzipo *et al.*, 2000). In the Middle East, the intermediate form of *Fasciola* was reported from the Gilan Province in the north of Iran. Sahba *et al.* (1972) reported the first three *Fasciola* species as *F. hepatica*, *F. gigantica*, and *F. indica* by using morphometric analysis. Since *Fasciola* species are different in transmission, epidemiology, phylogenic characteristics and intermediate host of lymnaeid snails, differentiation of *Fasciola* species is of great importance in prevention of the outbreak of fasciolosis (Massoud, 1974, Mas-Coma and Bargues, 1997, Bargues *et al.*, 2002). The morphologic characteristics of adult and eggs of *Fasciola* are influenced by the age of the helminth, host, and method of preparing the specimens (Ashrafi *et al.*, 2006). Cross-fecundation of both *F. hepatica* and *F. gigantica* produces hybrid type, which causes morphological mistakes with other species (Agatsuma *et al.*, 2001).

In Asia, especially in Iran, the prevalence of *Fasciola* species has an overlap. *F. hepatica* is mostly prevalent in the high and moderate areas, while *F. gigantica* exists in low land and tropical areas of the country (Agatsuma *et al.*, 2001). Furthermore, human fasciolosis is ecologically common in the areas with *F. hepatica* and *F. gigantica* overlap (Cheng and Bogitsh, 1998). Thus, the present study was performed to compare morphologic and morphometric characteristics of adult helminths and eggs of *Fasciola* species from domestic ruminants of northwestern Iran.

Materials and methods

Collection of fasciolid helminths

The livers of the ruminants (130 breeds of cattle, 67 buffaloes and 140 sheep) were collected from Urmia industrial slaughterhouse, and were transferred to Parasitology laboratory. The inflected livers were dissected and the helminths were extracted. They were washed several times in 0.01M phosphate buffer saline (PBS, pH=7.2), were stained using Asetokarmin, and were examined under light microscope at 100× magnification. *Fasciola* species were identified using key identification described by Soulsby (1982) and Moghaddam *et al.* (2004). The *Fasciola* eggs were directly extracted from the uterus of adult helminths by using dissecting needle and were washed within 0.01M PBS (pH=7.2). The eggs were incubated at +4°C until examination.

Fasciolid forms and eggs examination

A total number of 300 *Fasciola* species, i.e. 150 *F. hepatica* from cattle and 150 *F. gigantica* from water buffaloes were examined using optic microscopy and then were photographed. The all examined *Fasciola* species were also compared with donated specimens of *F. hepatica* and *F. gigantica* which were molecularly standard by Yakhchali *et al*. (2015) (Table1). To determine the size of fasciolid helminths, the distance between the suckers (DBS), the distance between the ventral sucker and the posterior end of body (DBVE), body length (L), body width (W), and proportion of body length to width (SI) were considered. To find out egg morphology and morphometry, a total number of 100 *Fasciola* eggs from cattle and sheep were individually measured and recorded using an Olympus compound microscope with an ocular micrometer at 1000×.

Table l. The prevalence of *Fasciola* species from the examined ruminants of northwestern Iran (n=337).

Animals	No. of examined animals	Prevalence (*n/N*, %)	No/H (%)	*Fasciola* species		
				Fh	Fg	InF
Cattle	120	30.7	9.23	8.94	23.99	17.93
Water buffaloes	67	34	6.85	4.91	9.96	4.58
Sheep	140	22.8	5	2.97	17.93	8.77
Total	337	28.19	7	16.83	51.89	31.28

Notes: Fh, *Fasciola hepatica*; Fg, *Fasciola gigantica*; InF, Intermediate form of *Fasciola*; No/H, number of helminths per each animal.

Statistical analysis

The body size and measurements of eggs were analyzed by SPSS statistical program (version 14, SPSS Inc., Chicago, Illinois, USA) using two sample *t*-tests and One-Way ANOVA. A probability of ≤ 0.05 was regarded as significant.

Results

The overall prevalence was 28.19% with the highest infection rate in water buffaloes (34%). *Fasciola gigantica* (51.89%) was found to be a prevalent specie in the examined ruminants. The highest number of *Fasciola* per each animal was recorded for cattle (9.23%) with diversity of *F. hepatica* (8.94%), *F. gigantica* (23.99%), and intermediate form (17.93%).

Morphologic and morphometric variations of adult flukes

F. hepatica was leaf-shaped with an oblique body angle, an evident shoulder and a cephalic cone and an oral sucker with a short distance from ventral sucker in the upper one-third of the body. In *F. gigantica* and intermediate form, cephalic cone was evident without a shoulder. The number of caecum branches particularly in the internal edge was more than the *F. hepatica*. The ovary and uterus branches in *F. gigantica* were also longer than that of *F. hepatica*.

The micrometry findings of adult fluke are presented in Table 2. The L, W, DBVE, and SI of all three adult *Fasciola* from all of the examined ruminants had significant differences with exceptions for SI and DBS of *F. gigantica* from water buffaloes origin (p=0.0001) and DBS of intermediate form of *Fasciola* from all of the examined animals. The W of intermediate form of *Fasciola* from water buffaloes origin had a significant difference with cattle and sheep origins. The L had also a significant difference between *F. gigantica* and intermediate form from cattle and water buffaloes origins (p<0.05).

Table 2. The body features of identified *Fasciola* species from the examined ruminants of northwestern Iran (Mean±SD).

Animals	*Fasciola* species	L (cm)	W (cm)	DBVE (cm)	DBDV (cm)	SI (cm)
Cattle	Fh	0.24±2.65	0.12^a± 1.36	0.25±2.34	0.06±0.26	0.13^a± 1.93
	Fg	0.29±2.90	0.12^a± 0.78	0.31±2.59	0.06± 0.26	3.73±0.53^a
	InF	^{a} 0.20±2.52	0.11^a± 1.01	^{a} 0.22±2.254	0.06±0.26	2.5±0.23^a
Water buffaloes	Fh	0.30± 1.62	0.14^a± 0.87	0.28±1.36	0.05±0.23	1.85±0.16^a
	Fg	0.45^a± 2.87	0.11±0.78	^{a} 0.46±2.62	0.04±0.24	3.64±0.32^a
	InF	0.30± 1.72	0.17^a± 0.69	1.44^b±0.29	0.049±0.25	2.42±0.21^a
Sheep	Fh	0.19^a± 2.15 0.41^a± 3.36	0.12^a± 1.13	^{a} 0.21±1.89	0.076±0.24	1.9±0.16^a
	Fg	0.26^a± 2.75	0.097^a± 0.91	3.07±0.42^a	0.064±0.26	3.66±0.35^a
	InF		^{a} 0.10±1.037	2.48±0.27^a	0.04±0.24	2.66±0.19^a

Notes: DBDV, distance between dorsal and ventral sucker, DBVE, the distance between ventral sucker and the posterior end of the body; L, body length; SI, proportion of body length to width. (^{a}Significant p<0.05)

Table 3. The features of identified *Fasciola* species eggs from the examined ruminants of northwestern Iran (Mean±SD).

Animals	*Fasciola* species	L (cm)	W(cm)	SI (cm)
Cattle	Fh	15.16^a±242.3	14.43^a± 135.37	0.23±1.78
	Fg	15.32^a±229.93	13.53^a± 130.77	0.14^a± 1.76
	InF	14.6±234.53	11.88±134.94	0.16±1.73
Sheep	Fh	233.88±19.92^a	141.84±16.09	1.65±0.16
	Fg	16.29^a± 230.73	12.76±138.16	0.15±1.67
	InF	17.87^a± 236.73	16.59±143.58	0.17±1.62

The eggs' morphology and morphometery variations

The eggs of *Fasciola* species were yellow light brown and oval with operculum in the end point. There was undistinguished cellular mass surrounded by a great number of yellow seeds inside the eggs. The seeds were in the middle line of the eggs near to operculum.

The results of micrometry of the eggs from all *Fasciola* species were shown in Table 3. There was a significant difference between L, W and SI of eggs of *F. hepatica* from sheep and cattle (p<0.05). The L, W and SI of eggs from all three species of *Fasciola* had no significant differences (p>0.05). The L and SI of eggs of *F. hepatica* and the intermediate form of *Fasciola* from sheep and goats had significant differences (p<0.05).

Discussion

Fasciola species are remarkable liver flukes of ruminants in all areas of Iran which yearly impose direct and indirect economic losses (Moghaddam *et al.*, 2004). In the present study, water buffaloes had the highest prevalence. However, the highest number of helminths per each animal was recorded for the cattle. *F. gigantica* was found to be a prevalent specie in the examined ruminants in northwestern Iran. The infections with both *F. hepatica* and *F. gigantica* have been reported from ruminants of different parts of Iran (Salahi-Moghaddam, 2004, Yakhchali and Ghobadi, 2005, Ashrafi *et al.*, 2006).

The morphologic findings of *Fasciola* species from the examined ruminants were nearly identical to the reports from other parts of Iran and the world. In Asia, *F. hepatica* and *F. gigantica* have overlaps in different areas, i.e. Iran, Japan, Pakistan, Korea, Taiwan and the Philippines (Mas-Coma and Bargues, 1997). All three forms of *Fasciola* have been reported in Egypt and Japan (Terasaki *et al.*,

1982, Esteban *et al.*, 1998). Regardless of diploid, triploid and/or mixoploidy forms of *Fasciola*, Agastuma *et al.* (1994) noted *Fasciola* reproduction because of abnormal spermatogenesis through parthenogenesis led to the formation of three different genotypes including genotype I (*F. hepatica*-like), genotype II (*F. gigantica*-like), and genotype III (intermediate form). Genetically, genotype I was absolutely distinctive from genotype II and genotype III. In different parts of Iran, particularly, in low lands and mountainsides the overlap of *Fasciola* species and the coinfection infection of the liver were reported (Ashrafi *et al.*, 2006). Recently, an intermediate form of *Fasciola* has been reported from ruminants of Fars Province in south and Mazandaran Province in the north of Iran (Salahi-Moghaddam, 2004, Karimi, 2008).

The morphometric evaluation of helminths revealed differences among *Fasciola* species from the examined ruminants. The L of *F. hepatica* from water buffaloes origin was longer than other *Fasciola* species from cattle and sheep while, the L of *F. hepatica* and the intermediate form of *Fasciola* from water buffaloes origin was respectively longer than the specimens from sheep and cattle origins. Micrometric or even morphological evaluation of body features of *Fasciola* species may be influenced by final host, immunity of host, intermediate host or various genealogy factors (Dalton, 1999). Sahba (1972) noted that the L of *F. gigantica* (4.39-5.2 cm) was longer than *F. hepatica* (1.88-2.33 cm) and another fasciolid with size L in between them was reported as *F. indica*. Mayne (2000) also reported that the average body size of *F. hepatica* (1-2×2-3 cm) was less than that of *F. gigantica* (0.5-1.3×2.4-7.6cm).

The SI of *F. gigantica* and the intermediate form from the examined animals was more than *F. hepatica*. This finding was in agreement with Malek (1981) and Valero *et al.* (2001). According to morphometric findings of *Fasciola* species from the ruminants of northern Iran, the SI for *F. hepatica*, *F. gigantica* and intermediate form from sheep origin was 1.90, 4.20, 2.61, and for cattle and water buffaloes the origins were 1.96, 3.46, and 2.2 respectively. In a genomic study by Agatsuma *et al.* (2000), it was shown that there was cross-hybridization between both *F. hepatica* and *F. gigantica*. Ashrafi *et al.* (2006) reported that the intermediate form of *Fasciola* from Iran was originally different from the other reports elsewhere. Bargues *et al.* (2002) confirmed the existence of intermediate fasciolid form of *F. hepatica* and *F. gigantica* in Iran. In a descriptive study considering the features of the cephalic cone, L, W, SI, area (A), and perimeter (P) on typical and atypical fasciolids using Computerized Image Analysis System (CIAS), Salahi-Moghaddam (2004) reported that they were morphologically the intermediate forms of *Fasciola*. According to Ashrafi *et al.* (2006) the intermediate form of *Fasciola* was different from other reports. Salahi-Moghaddam (2004) studied atypical helminths beside the typical fasciolids descriptively and confirmed the intermediate form with regard to characteristics such as cephalic cone and SI. Ashrafi *et al.* (2006) elucidated that *F. hepatica* was larger than the classic form of *F. hepatica*. However, *F. gigantica* was slightly narrower with lesser A and larger sizes than the standard forms from Burkina Faso. The two *Fasciola* species, i.e. *F. california* and *F. halli* were reported according to evolutionary features, geographical distribution and tegument shape from American ruminants (Sinitsin, 1993). In addition, Ali (1993) morphologically reported intermediate forms of *F. hepatica* and *F. gigantica* from Egypt.

The morphology of *Fasciola* species eggs in the present study was in agreement with earlier reports (Soulsby, 1982, Scmidt and Roberts, 2000, Eslami, 2008). The eggs of *F. hepatica* were ovoid, bile-stained and operculated. The L, W and SI of measured eggs of *Fasciola* species were nearly similar. There was a significant difference between the length of *F. hepatica* and *F. gigantica* eggs. The average egg sizes of *F. hepatica* and *F.*

gigantica from Iranian ruminants were 60-92 by 128-152µm and 68-94 by 135-190µm, respectively (Salahi-Moghaddam, 2004). In previous reports, the length of eggs of *F. hepatica* was reported to be less than eggs of *F. gigantica* (Soulsby, 1982, Mayne, 2000). According to Scmidt and Roberts (2000), the measure of *F. hepatica* eggs was 63-90×130-150µm in size while *F. gigantica* eggs was larger and was measured to be 70-90 by 160-190µm. The size of *F. gigantica* eggs was 90-104 by 156-197µm and the F. *hepatica* eggs were of size 63-90 by 130-150µm (Soulsby, 1982). Paniker (1997) reported that *F. hepatica* eggs were about 80 by 140µm in size. Parija (1996) also reported egg size of *F. hepatica* was 63-90 by 130-150µm.

From the results of the present study, the prevalence of *Fasciola* was indicative in examined ruminants of northwestern Iran. In addition, cattle play an important role as a source of infection for other definitive hosts to which different *Fasciola* forms co-exist in the ruminants of the region. The morphology and micrometery findings may also be taxonomically informative and reliable to discriminate morphologically and morphpmetericaly *Fasciola* species from different animal origins. These findings were of great importance to humans and ruminants because of the differences in transmission and epidemiology characteristics. Thus, further studies were recommended to be carried out in order to determine the polymorphism of *Fasciola* species from lymnaeid snails and final hosts.

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مطالعه ریخت شناسی و مورفومتریک تخم و کرم بالغ گونه های *فاسیولا* انگل کبد نشخوارکنندگان اهلی شمال غرب ایران

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دریافت مقاله: 1393/1/21 پذیرش نهایی: 1395/5/23

چکیده

گونه های *فاسیولا* عامل فاسیولوزیس انسان و دام در ایران و جهان می باشند که تا حدودی ریخت شناسی مشابهی دارند. مطالعه حاضر به منظور شناسایی و مقایسه ویژگی های مورفولووژیک و مورفومتریک گونه های *فاسیولا* انگل نشخوارکنندگان اهلی در شمال غرب ایران انجام شد. تعداد 130، 67 و 140 عدد کبد به ترتیب از گاو، گاومیش و گوسفند جمع آوری شدند. پس از برش دادن کبد کرم بالغ *فاسیولا* جمع آوری و رنگ آمیزی استوکارمین شدند. فراوانی کلی آلودگی در نشخوارکنندگان 28/19درصد بود. بیشترین آلودگی در گاومیش (34درصد) و بیشترین نسبت تعداد کرم بالغ *فاسیولا* به هر راس دام در گاو (9/23) بود. از نظر تنوع گونه ای *فاسیولا ژیگانتیکا* (51/89درصد) گونه شایع جدا شده از گاومیش بود. مقایسه میانگین طول، عرض و فاصله بادکش شکمی تا دم در اشکال مختلف *فاسیولا* جدا شده از گاو، گاومیش و گوسفند اختلاف معنی داری داشت. میانگین طول در گونه های *فاسیولا* انگل گاو و گاومیش در *فاسیولا* حد واسط و *فاسیولا ژیگانتیکا* جدا شده از گاو و گاومیش اختلاف معنی داری بود. میانگین عرض و نسبت طول به عرض تخم در گونه های مختلف *فاسیولا* اختلاف معنی داری نداشت. اختلاف میانگین طول تخم *فاسیولا هپاتیکا و فاسیولا ژیگانتیکا* معنی دار بود. نتایج حاصل از این مطالعه نشانگر حضور هر سه شکل *فاسیولا* در نشخوارکنندگان شمال غرب ایران بود. به علاوه، یافته های ریخت شناسی و مورفومتری تخم و کرم بالغ گونه های *فاسیولا* در میزبان های مختلف نشان داد که تمایز آنها از نظر تاکسونومیک راهنما و میسر می باشد.

واژگان کلیدی: *فاسیولا*، تخم، نشخوارکننده، ایران.